

Marked-up Version of Amended Specification and Claims Pursuant to 37 C.F.R. §§ 1.121(b)-(c)

In the specification:

On page 1, after the title, please insert the following new paragraph:

Priority Claim

This is a divisional of U.S. Patent Application No. 09/423,233 filed June 27, 2000, which is a § 371 national stage of PCT/US98/08926 filed May 1, 1997, in turn claims the benefit of U.S. Provisional Application No. 60/045,400 filed May 2, 1997.

Please add the following abstract as page 44 of the specification:

NUCLEIC ACIDS FOR DETECTING ASPERGILLUS AND OTHER FILAMENTOUS FUNGI

Abstract

Nucleic acids for detecting Aspergillus species and other filamentous fungi are provided.

Unique internal transcribed space 2 coding regions permit the development of nucleic acid probes specific for five different species of Aspergillus, three species of Fusarium, four species of Mucor, two species of Penecillium, five species of Rhizopus, one species of Rhizomucor, as well as probes for Absidia corymbifer, Cunninghamella elagans, Pseudallescheria boydii, and Sporothrix schenkii. Methods are disclosed for the species-specific detection and diagnosis of infection by Aspergillus, Fusarium, Mucor, Penecillium, Rhizomucor, absidia,

Cunninghaemella, Pseudallescheria or Sporthrix in a subject. Furthermore, genus-specific probes are also provided for Aspergillus, Fusarium and Mucor, in addition to an all-fungus nucleic acid probe.

In the claims:

Please amend the claims as follows:



(Amended) [The isolated nucleic acid probe of claim 51] An isolated nucleic acid 1. probe that consists essentially of 10 to 50 consecutive nucleotides for species-specific identification of Fusarium, wherein the probe hybridizes to the internal transcribed spacer 2 nucleic acid sequence [is selected from the group consisting] of [Aspergillus flavus (SEQ ID NO:1), Aspergillus fumigatus (SEQ ID NO:2), Aspergillus niger (SEQ ID NO:3), Aspergillus terreus (SEQ ID NO:4), Aspergillus nidulans (SEQ ID NO:5)], Fusarium solani (SEQ ID NO:6) [,] or Fusarium moniliforme (SEQ ID NO:7), but does not hybridize to the internal transcibed space 2 nucleic acid sequence of Aspergillus flavus (SEQ ID NO:1), Aspergillus fumigatus (SEQ ID NO:2), Aspergillus niger (SEQ ID NO:3), Aspergillus terreus (SEQ ID NO:4), Aspergillus nidulans (SEQ ID NO:5), Mucor rouxii (SEQ ID NO:8), Mucor racemosus (SEQ ID NO:9), Mucor plumbeus (SEO ID NO:10), Mucor indicus (SEO ID NO:11), Mucor circinilloides f. circinelloides (SEQ ID NO:12), Rhizopus oryzae (SEQ ID NO:13 and NO:14), Rhizopus microsporus (SEQ ID NO:15 and 16), Rhizopus circinans (SEQ ID NO:17 and 18), Rhizopus stolonifer (SEQ ID NO: 19), Rhizomucor pusillus (SEQ ID NO:20), Absidia corymbifera (SEQ ID NO:21 and 22), Cunninghamella elegans (SEQ ID NO:23), Pseudallescheria boydii (teleomorph of Scedosporium apiospermum) (SEQ ID NO:24, 25, 26, and 27), Penicillium notatum (SEQ ID NO:28), or Sporothrix schenkii (SEQ ID NO:29) [wherein the probe selectively hybridizes to a portion of at least one of the nucleic acid of SEQ ID NOS:1-29, or a complementary sequence thereof, respectively].

Please cancel claims 2-6.

- 7. (Amended) The isolated nucleic acid probe of Claim 1[capable of] wherein the probe selectively [hybridizing] hybridizes with a *Fusarium solani* nucleic acid of SEQ ID NO:6, or a complementary sequence thereof.
- 8. (Amended) The isolated nucleic acid probe of Claim 1 [capable of] wherein the probe selectively [hybridizing] hybridizes with a Fusarium moniliforme of SEQ ID NO:7, or a complementary sequence thereof.

Please cancel claims 9-23.

24. (Twice amended) A method of detecting a species of [filamentous fungi selected from the group consisting of Aspergillus [flavus (SEQ ID NO:1), Aspergillus fumigatus (SEQ ID NO:2)], Aspergillus nigerSEQ ID NO:3), Aspergillus terreus (SEQ ID NO:4), Aspergillus nidulans

(SEQ ID NO:5),]Fusarium solani (SEQ ID NO:6) or Fusarium moniliforme (SEQ ID NO:7), [Mucor rouxii (SEQ ID NO:8), Mucor racemosus (SEQ ID NO:9), Mucor plumbeus (SEQ ID NO:10), Mucor indicus (SEQ ID NO:11), Mucor circinilloides f. circinelloides (SEQ ID NO:12), Rhizopus oryzae (SEQ ID NO:13 and NO:14), Rhizopus microsporus (SEQ ID NO:15 and 16), Rhizopus circinans (SEQ ID NO:17 and 18), Rhizopus stolonifer (SEQ ID NO: 19), Rhizomucor pusillus (SEQ ID NO:20), Absidia corymbifera (SEQ ID NO:21 and 22), Cunninghamella elegans (SEQ ID NO:23), Pseudallescheria boydii (teleomorph of Scedosporium apiospermum) (SEQ ID NO:24, 25, 26, and 27), Penicillium notatum (SEQ ID NO:28), or Sporothrix schenkii (SEQ ID NO:29)] in a sample comprising

contacting the sample with a nucleic acid probe consisting essentially of 10 to 50 consecutive nucleotides that selectively hybridizes with [at least one of] a nucleic acid [of SEQ ID NO:1-29] having a sequence as set forth as SEQ ID NO:5 or SEQ ID NO:6, or a complementary sequence thereof[, respectively];

wherein hybridization of the nucleic acid probe with the sample indicates the detection of the *Fusarium* species in the sample.

Please cance claims 25-29.

- 30. (Amended) The method of Claim 24, wherein the probe [is capable of] selectively [hybridizing] <u>hybridizes</u> with a *Fusarium solani* nucleic acid of SEQ ID NO:6, or a complementary sequence thereof.
- 31. (Amended) The method of Claim 24, wherein the probe [is capable of] selectively [hybridizing] <u>hybridizes</u> with a *Fusarium moniliforme* of SEQ ID NO:7, or a complementary sequence thereof.

Please cancel claims 34-46.

47. (Amended) An isolated nucleic acid probe for identifying a member of a [genus selected from the group consisting of *Aspergillus*,] Fusarium genus [and Mucor] wherein the probe [selectively hybridizes to a portion of a nucleic acid having a sequence as set forth as of SEQ ID



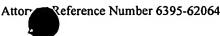
NOS:58-60,] consists essentially of a nucleotide sequence as set forth as SEQ ID NO:59, or a complementary sequence thereof, respectively.

Please cancel claim 48.

49. (Amended) A method for detecting a member of a [genus selected from the consisting of Aspergillus,] Fusarium genus [and Mucor] in a sample, comprising combining the sample with a nucleic acid probe [capable of] that selectively [hybridizing] hybridizes with a portion of the nucleic acid of [of SEQ ID NOS:58-60] SEQ ID NO:59, or a complementary sequence thereof, respectively, [the presence of hybridization indicating the detection of the respective genus] wherein hybridization of the probe with the sample indicates the presence of Fusarium in the sample.

Please add the following new claims:

51. (New) An isolated nucleic probe that hybridizes to an internal transcribed spacer 2 region of a Fusarium species, wherein the probe consists essentially of a nucleic acid sequence having a sequence as set forth as SEQ ID NO:49, SEQ ID NO:50, or SEQ ID NO:51, and wherein the probe does not hybridize to but does not hybridize to the internal transcribed space 2 nucleic acid sequence of Aspergillus flavus (SEQ ID NO:1), Aspergillus fumigatus (SEQ ID NO:2), Aspergillus niger (SEQ ID NO:3), Aspergillus terreus (SEQ ID NO:4), or Aspergillus nidulans (SEQ ID NO:5), Mucor rouxii (SEQ ID NO:8), Mucor racemosus (SEQ ID NO:9), Mucor plumbeus (SEQ ID NO:10), Mucor indicus (SEQ ID NO:11), Mucor circinilloides f. circinelloides (SEQ ID NO:15 and 16), Rhizopus oryzae (SEQ ID NO:13 and NO:14), Rhizopus stolonifer (SEQ ID NO:15 and 16), Rhizopus circinans (SEQ ID NO:17 and 18), Rhizopus stolonifer (SEQ ID NO:19), Rhizomucor pusillus (SEQ ID NO:20), Absidia corymbifera (SEQ ID NO:21 and 22), Cunninghamella elegans (SEQ ID NO:23), Pseudallescheria boydii (teleomorph of Scedosporium apiospermum) (SEQ ID NO:24, 25, 26, and 27), Penicillium notatum (SEQ ID NO:28), or Sporothrix schenkii (SEQ ID NO:29).



- (New) The isolated nucleic probe of Claim 1, wherein the probe consists 52. essentially of a nucleic acid sequence having a sequence as set forth as SEQ ID NO:49.
- (New) The isolated nucleic probe of Claim 1, wherein the probe consists 53. essentially of a nucleic acid sequence having a sequence as set forth as SEQ ID NO:50.
- (New) The isolated nucleic probe of Claim 1, wherein the probe consists essentially of a nucleic acid sequence having a sequence as set forth as SEQ ID NO:51.
- 55. (New) The method of Claim 24, wherein the probe consists essentially of a nucleic acid sequence having a sequence as set forth as SEQ ID NO:49.
- (New) The method of Claim 24, wherein the probe consists essentially of a 56. nucleic acid sequence having a sequence as set forth as SEQ ID NO:50.
- (New) The method of Claim 24, wherein the probe consists essentially of a nucleic acid sequence having a sequence as set forth as SEQ ID NO:51.
- 58. (New) A isolated nucleic acid sequence comprising a sequence as set forth as SEQ ID NO:5 or SEQ ID NO:6.
- 59. (New) A isolated nucleic acid sequence consisting essentially of a sequence as set forth as SEQ ID NO:5 or SEQ ID NO:6.